

COMPARATIVE EVALUATION OF TOOTHPASTE WITH DIFFERENT FORMULATION IN REDUCING STREPTOCOCCUS MUTANS- AN INVITRO STUDY

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ABSTRACT--Salivary microfloras like *Streptococcus mutans* and other predisposing factors lay an important role in the initiation and progression of dental diseases such as dental caries .Toothpaste have been formulated in such a manner that it should improve the oral health, control plaque formation, bacteria and candida colonisation. The chemical agent used may have unsatisfactory effects such as tooth staining, taste alterations and hypersensitivity reactions . The organism *Streptococcus mutans* was isolated from sample of saliva using special media and maintained in Tryptose soya agar at 4 degree C in the department of microbiology, Saveetha dental college and hospitals.According to the results of the present study, herbal toothpaste can cause inhibition of bacterial growth. The organisms employed in the present study include both the normal flora and the pathogens of the oral cavity. *S.mutans* has been strongly associated with the initiation of caries, while there is a correlation between *Lactobacilli* and the further development of carious lesions. The herbal toothpaste formulations studied in our experiments, appear to be more effective than the fluoride dental formulations. Hence, herbal toothpaste has been proven to have superior antimicrobial activity against *streptococcus mutans* than the fluoridated toothpaste

Key words -- *Streptococcus mutans*, tooth paste, *lactobacilli*, antimicrobial, dental caries

I. INTRODUCTION

Over the past years, pharmaceutical companies are attentive in inspecting plants as sources for new phytotherapeutic agents with proven efficacy, safety and quality (1) . The extent of drug resistant pathogens is one of the most serious threats to successful treatment of microbial diseases (2) .Salivary microfloras like *Streptococcus mutans* and other predisposing factors lay an important role in the initiation and progression of dental diseases such as dental caries (3) .Dental caries also known as tooth decay is the most common occurring diseases of people worldwide caused due to the breakdown of the enamel. Dental caries is seen mostly in children of age group of 6-11 years and in adolescents between 12 to 19 years (4) .*Streptococcus mutans*, an acidogenic and aciduric microorganism colonizing the oral cavity, is considered to be the main cause of dental caries (5).Risk for caries includes physical, biological, environmental, behavioural, and lifestyle-related factors such as high numbers of

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cariogenic bacteria, inadequate salivary flow, insufficient fluoride exposure, poor oral hygiene, inappropriate methods of feeding infants, and poverty (6). The eradication of carcinogenic bacteria from the oral cavity using antibacterial agent is the primary strategy in prevention of dental caries (7).

India is well known as the gold mine of well-recorded and well-practised knowledge of traditional herbal medicine. Herbal medicine is considered as the backbone, in which about 75-80% of them are time tested and distributed all over the world population, mainly in the developing countries primarily in India (8). Herbs are natural products and their composition differs from person to person and from energetic decoctions to the use of herbal extracts following Western methodologies of general medicine. Traditional herbal medicine has a long history, it is the sum total of the practices based on the theories, beliefs and experiences of different cultures and times, often inexplicable, used in the maintenance of health, as in the prevention, diagnosis, improvement and treatment of illnesses (9).

Herbal extracts like chamomile, Ocimum, and Echinacea when used topically provide therapeutic benefits in the oral cavity (10). The benefit of using plant derived medicinal preparations such as poultices, infusions and simple extracts or powders of dried seeds and roots, for curing of oral diseases, have been used as endemic systems of medicine since olden times (11). The common ingredient present in the herbal toothpaste are calcium carbonate, water, glycerin, Sodium Lauryl Sulphate, Extracts of Ginger, Camphor, Menthol & Clove oil, Sodium Carboxymethyl Cellulose, Silica, Sodium Benzoate & Sodium Saccharine, Sorbitol (2). Ingredients used in modern toothpaste formulations include abrasive agents, tensioactives, humectants, thickening agents, flavoring, coloring agents and antimicrobial agents. These antimicrobial agents include metal salts, phenols, herbal extracts, enzymes, essential oils, and bis biguanides (12). Toothpaste have been formulated in such a manner that it should improve the oral health, control plaque formation, bacteria and candida colonisation. The chemical agent used may have unsatisfactory effects such as tooth staining, taste alterations and hypersensitivity reactions (13). Neem which is an active ingredient in herbal toothpaste formulation is said to have antibacterial activity. It has been used for the various activities like as an astringent, antiseptic, insecticidal, anti ulcer and for cleaning the teeth in pyorrhea and other dental disease (14). The other important ingredient used is clove, many of them use it as a spice and also unaware of its health beneficial activity. The clove contain an anesthetic substance called Eugenol which will reduce infection and relieve pain. Aloe Vera gel has been reported to smooth gum tissue and relieve pain and discomfort when applied on gums (7),(15).

In the present study we have evaluated the efficacy of eight herbal toothpaste with different formulation in reducing streptococcus mutans.

II. MATERIALS AND METHODS

The organism *Streptococcus mutans* was isolated from sample of saliva using special media (*Mutans-sanguis* agar which is recommended for differentiation of *Streptococcus mutans* and *Streptococcus sanguis* associated with oral microflora) and maintained in Tryptose soya agar at 4 degree C in the department of microbiology, Saveetha dental college and hospitals (16).

Eight commercially available herbal toothpastes and its main composition: (i) Herbal Toothpaste 1 - calcium carbonate and sorbitol (ii) Herbal Toothpaste 2 - lavang, dasanakanti churnam (iii) Herbal Toothpaste 3 - Piper

nigrum, pippali (iv)Herbal Toothpaste 4 - Sodium Lauryl sulphate, zinc oxide (v)Herbal Toothpaste 5 - Neem extract , hydrated Cilica (vi)Herbal Toothpaste 6 - Alovera, Salvadora persica (vii) Herbal Toothpaste 7-patang, lavang (viii)Herbal Toothpaste 8- Calcium carbonate, glycerin Control – Fluoridated toothpaste. Pour plate method was used for screening the antibacterial activity.

III. METHODOLOGY

Pour plate technique was followed for screening the activity of different herbal toothpastes in reducing Streptococcus mutans count. 1 g of different herbal toothpastes were diluted in 1 ml of saline and mixed with Mutans–sanguis agar and the plates and allowed to set. Broth culture of the bacterial strain, Streptococcus mutans compared to Mcfarland standard 0.5 was prepared. Lawn culture of the test organisms were made on the agar plates using sterile cotton swab and the plates were dried for 15 minutes. The plates were incubated at 37°C overnight and the bacterial growth was recorded as number of colony forming units. All the tests were done in triplicate to minimize the test error.

IV. RESULT AND DISCUSSION

The results are tabulated in table 1. All the tooth pastes with different herbal formulation as described earlier has got inhibitory activity on oral pathogens

Table 1 : Colony forming units on culture plates with different tooth pastes

TOOTHPASTE	CFU/ml
herbal toothpaste 1	70
herbal toothpaste 2	20
herbal toothpaste 3	nil
herbal toothpaste 4	nil
herbal toothpaste 5	10
herbal toothpaste 6	30
herbal toothpaste 7	nil
herbal toothpaste 8	60
fluoridated toothpaste	50

Periodontal diseases encompass multifactorial diseases involving bacterial biofilms and the generation of an inflammatory response, including the production of cytokines, eicosanoids, and matrix metalloproteinase.

Bacterial biofilms have been proven to be the primary etiological factor in the initiation of gingivitis and subsequent destruction of periodontal tissues (17). It is well established that supragingival plaque is the cause of gingivitis and plays a primary role in the initiation of periodontitis. The removal of microbial plaque leads to resolution of gingival inflammation, and cessation of plaque control leads to a recurrence of inflammation. The control of plaque in the maintenance of gingival health has been well established in the literature. It has been shown that rigorous self-performed plaque control over long periods of time reduced the levels and altered the composition of sub gingival bacteria and reduced the frequency of deep periodontal pockets (18).

Since the 1980's fluoride has been the most commonly used remineralizing agents (19). It is known to control caries predominantly through its topical effect. Fluoride inhibits demineralization, enhanced remineralization, and inhibits bacterial activity. When the acid attacks the enamel surface, the pH begins to rise and fluoride present in the microenvironment causes enamel dissolution to stop. It acts by creating phases of calcium and phosphate thereby, increasing the surface fluoride content in the enamel. The other contributing factors for fluoride is its antimicrobial property, reduction in bacterial adherence and increases the plaque pH (20). Thus, The fluoride toothpaste reduces the number of streptococcal colonies despite the fact that fluoride was added to the toothpastes first with the aim to preserve the product and then to protect the teeth from caries (2). The effectiveness of fluoride toothpastes as an antimicrobial agent is concentration dependent.

According to the results of the present study, herbal toothpaste can cause inhibition of bacterial growth. The organisms employed in the present study include both the normal flora and the pathogens of the oral cavity. *S.mutans* has been strongly associated with the initiation of caries, while there is a correlation between *Lactobacilli* and the further development of carious lesions (21). It was observed that the dentifrice with multiple herbal components is more effective in inhibiting both the organisms as herbal extracts have received special attention because of being non-chemical and non-synthetic in nature, and have been used in traditional medicine (22). The antimicrobial activity of the herbs is due to the presence of secondary metabolites such as alkaloids, flavonoids, polyphenols, and lectins. Synergistic interactions between the principal components of these herbal products is considered as a vital part of their efficacy (23). Our study mainly concentrates in comparing efficacy of herbal dentifrices with conventional ones in inhibiting cariogenic bacteria, suggesting that the efficacy of herbal dentifrices is similar and have superior antibacterial efficacy (24). Hence, the addition of natural plant extracts to toothpastes can increase the antimicrobial spectrum, thus reducing, controlling or preventing oral diseases (25).

V. CONCLUSION

In conclusion, the herbal toothpaste formulations studied in our experiments, appear to be more effective than the fluoride dental formulations. Hence, herbal toothpaste has been proven to have superior antimicrobial activity against streptococcus mutans than the fluoridated toothpaste due to the excess use of fluoride that can cause dental fluorosis, stomach ailments, acute toxicity, skin rashes (perioral dermatitis) these herbal products are a symbol of safety in contrast to synthetic toothpaste; that are regarded as unsafe to human beings and environment. Inhibit plaque may be expected to be of value in both the prevention and management of periodontal disease thus, exhibiting major effect on improving the oral health of the individual. However, further studies are needed to know the efficacy of these toothpastes.

REFERENCES

1. Groppo, F.C., Bergamaschi, C.D.C., Cogo, K., Franz-Montan, M., Motta, R.H.L. and Andrade, E.D.D., 2008. Use of phytotherapy in dentistry. *Phytotherapy research*, 22(8), pp.993-998
2. Kurian, M. and Geetha, R.V., 2015. Effect of herbal and fluoride toothpaste on *Streptococcus mutans*-a comparative study. *Journal of pharmaceutical sciences and research*, 7(10), p.86
3. Mehta, S., Pesapathy, S., Joseph, M., Tiwari, P.K. and Chawla, S., 2013. Comparative evaluation of a herbal mouthwash (Freshol) with chlorhexidine on plaque accumulation, gingival inflammation, and salivary *Streptococcus mutans* growth. *Journal of International Society of Preventive & Community Dentistry*, 3(1), p.25
4. Botelho, M.A., Nogueira, N.A.P., Bastos, G.M., Fonseca, S.G.C., Lemos, T.L.G., Matos, F.J.A., Montenegro, D., Heukelbach, J., Rao, V.S. and Brito, G.A.C., 2007. Antimicrobial activity of the essential oil from *Lippia sidoides*, carvacrol and thymol against oral pathogens. *Brazilian Journal of Medical and Biological Research*, 40(3), pp.349-356.
5. Patil, S., Venkataraghavan, K., Anantharaj, A. and Patil, S., 2010. Comparison of two commercially available toothpastes on the salivary streptococcus mutans count in urban preschool children-An in vivo study. *Group*, 1(50), p.53720.
6. Selwitz, R.H., Ismail, A.I. and Pitts, N.B., 2007. Dental caries. *The Lancet*, 369(9555), pp.51-59.
7. Vijayaalakshmi, L.G. and Geetha, R.V., 2015. Comparison of Herbal Mouth Wash with Conventional Mouth Wash in Use in Reducing *Streptococcus Mutans*-An Invitro Study. *Journal of Pharmaceutical Sciences and Research*, 7(7), p.485.
8. Kamboj, V.P., 2000. Herbal medicine. *Current science*, 78(1), pp.35-39.
9. Firenzuoli, F. and Gori, L., 2007. Herbal medicine today: clinical and research issues. *Evidence-Based Complementary and Alternative Medicine*, 4(S1), pp.37-40.
10. Somaraj, V., Shenoy, R.P., Shenoy Panchmal, G., Kumar, V., Jodalli, P.S. and Sonde, L., 2017. Effect of herbal and fluoride mouth rinses on streptococcus mutans and dental caries among 12–15-year-old school children: a randomized controlled trial. *International journal of dentistry*, 2017.
11. Jayashankar, S., Panagoda, G.J., Amaratunga, E.A.P.D., Perera, K. and Rajapakse, P.S., 2011. A randomised double-blind placebo-controlled study on the effects of a herbal toothpaste on gingival bleeding, oral hygiene and microbial variables. *Ceylon Medical Journal*, 56(1).
12. Adwan, G., Salameh, Y., Adwan, K. and Barakat, A., 2012. Assessment of antifungal activity of herbal and conventional toothpastes against clinical isolates of *Candida albicans*. *Asian Pacific journal of tropical biomedicine*, 2(5), pp.375-379.
13. Jenner, F., Jaleel, V.A., Kulshrestha, R., Maheswar, G., Rao, P.K. and Kranthi, J., 2013. Evaluating the antimicrobial activity of commercially available herbal toothpastes on microorganisms associated with diabetes mellitus. *J Contemp Dent Pract*, 14(05), pp.924-929.
14. Deshmukh, P., Telrandhe, R. and Gunde, M., 2017. Formulation and Evaluation of Herbal Toothpaste: Compared With Marketed Preparation. *Int J Pharm Drug Anal*, 5(10), pp.406-410.

15. Sarvesh Kumar J and Geetha. Effect of rosemary oil extract on biofilm formation by streptococcus mutans-
ainvitro study. International Journal of Current Advanced Research. Volume 6; Issue 4; April 2017; Page
No.3122-3123.
16. George, D., Bhat, S.S. and Antony, B., 2009. Comparative evaluation of the antimicrobial efficacy of Aloe
vera tooth gel and two popular commercial toothpastes: An in vitro study. Gen Dent, 57(3), pp.238-41.
17. Pradeep, A.R., Agarwal, E., Bajaj, P., Naik, S.B., Kumari, M. and Guruprasad, C.N., 2012. Clinical and
microbiological effects of commercially available dentifrice containing amine fluoride: A randomized
controlled clinical trial. Contemporary clinical dentistry, 3(3), p.265.
18. Buzalaf, M.A.R. ed., 2011. Fluoride and the oral environment (Vol. 22). Karger Medical and Scientific
Publishers.
19. Hemagaran, G. and Neelakantan, P., 2014. Remineralization of the tooth structure-the future of dentistry.
International Journal of PharmTech Research, 6(2), pp.487-493.
20. Mohankumar, K.P., Priya, N.K. and Madhushankari, G.S., 2013. Anti cariogenic efficacy of herbal and
conventional tooth pastes-a comparative in-vitro study. Journal of international oral health: JIOH, 5(2),
p.8.
21. Shaheen, S.S., Reddy, P., Hemalatha, S.R., Doshi, D., Kulkarni, S. and Kumar, M., 2015. Antimicrobial
efficacy of ten commercially available herbal dentifrices against specific oral microflora–In vitro study.
Journal of clinical and diagnostic research: JCDR, 9(4), p.ZC42.
22. Deshpande, R.R., Kachare, P., Sharangpani, G., Varghese, V.K. and Bahulkar, S.S., 2014. Comparative
evaluation of antimicrobial efficacy of two commercially available dentifrices (fluoridated and herbal)
against salivary microflora. Int J Pharm Pharm Sci, 6(6), pp.72-4.
23. Mohanavel, V. M. Karthick, D.L. Belginpaul. Fabrication and development of aluminum alloy AA6063-
titanium carbide composite prepared by in situ method, International Journal of Applied Engineering
Research, 10 (2015) 12475-12481.
24. Mohanavel, V. E. Arun Kumar, N. Devaraj, P. Kumar. Effect of boron carbide addition on impact behavior
of AA6360/Al₂O₃ hybrid composites fabricated by stir casting method, International Journal of Applied
Engineering Research, 10 (2015) 341-344
25. Adwan, G., Salameh, Y., Adwan, K. and Barakat, A., 2012. Assessment of antifungal activity of herbal
and conventional toothpastes against clinical isolates of Candida albicans. Asian Pacific journal of tropical
biomedicine, 2(5), pp.375-379.
26. Rossi, A.D., Ferreira, D.C.A., Silva, R.A.B.D., Queiroz, A.M.D., Silva, L.A.B.D. and Nelson-Filho, P.,
2014. Antimicrobial activity of toothpastes containing natural extracts, chlorhexidine or triclosan.
Brazilian Dental Journal, 25(3), pp.186-190.
27. Balagopal, S. and Arjunkumar, R., 2013. Chlorhexidine: The gold standard antiplaque agent. Journal of
Pharmaceutical sciences and Research, 5(12), p.270.